action of this antagonist is similar to that of fluorophenylalanine in poliovirus-infected cells, where the amino acid analogue inhibited the synthesis of poliovirus, but not the occurrence of cytopathology(18).

With the RNA-containing poliovirus, no inhibition of virus synthesis or development of cytopathology was observed at concentrations of amethopterin that inhibited DNA synthesis. Thus it appears that active cellular DNA synthesis is not required in the synthesis of a RNA-containing virus.

Summary. Amethopterin, a folic acid antagonist, has been found to prevent multiplication of HeLa cells in Eagle's medium containing added adenine and glycine. This inhibition can be removed by addition of thymidine or thymidylic acid. The amethopterin-induced thymine deficiency specifically results in a cessation of cell division and a disparate increase in cellular RNA and protein to DNA content. Ability of amethopterin-induced thymine deficient cultures to support viral synthesis appears to be dependent upon the type of nucleic acid contained in the virus. With DNA-containing viruses such as vaccinia and herpes simplex, viral replication is inhibited, whereas the synthesis of a RNA-containing virus such as poliovirus is not.

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Electrophoretic Studies of Serum Proteins and Glycoproteins During Early Stages of Experimental Tuberculosis.* (26090)

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Several studies have employed filter paper electrophoresis to determine changes in concentration of serum glycoproteins and proteins in clinical(1,2) and experimental(3) tuberculosis. Previous investigations have been primarily concerned with alterations in serum glycoprotein and protein patterns in well-established infections, and with the effects of therapy. The present study was undertaken to investigate the rate and extent of change in electrophoretic patterns in the early stages of an experimental infection.

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TABLE I. General, Pathologic and Hematologic Data.

Group (days post-infection)	No. guinea pigs	Index of tuberculosis†					Tuberculin reaction§	Hemoglobin (%)*	Hematocrit (%)*
		Spleen	Liver	Lungs	Lymph nodes	Total*			
0 (nor)‖	25							12.9 ±.10	41 ±.3
8	19	.5	.0	.0	.0	.5 ±.11	1.2	12.7 ±.14	40 ±.4
15	13	.6	.1	.0	1.7	2.4 ±.30	1.1	13.0 ±.21	43 ±.6‡
19	16	1.0	1.0	.1	1.5	3.6 ±.24	1.6	12.0 ±.18‡	39 ±.5‡
29	8	2.4	.8	.8	3.1	7.1 ±.41	4.0	11.8 ±.13‡	39 ±.5‡
50	18	2.0	1.5	1.8	2.6	7.9 ±.42	3.8	11.5 ±.18‡	41 ±.5

* Including stand. error of mean.

† Each organ was assigned a value of 0 to 4 according to extent of macroscopic tuberculosis. Maximum possible extent of disease in each animal was 16. Index of tuberculosis is avg total for each group.

‡ $P = <.01$.

§ Avg tuberculin reaction by rating of 0-4+.

‖ nor = normal.

Experimental. Adult, virgin guinea pigs with an average weight of 750 g were found to be nonreactors to intradermal injection of 0.1 ml of a 5% solution of Old Tuberculin. They were inoculated subcutaneously in the inguinal region with 0.1 mg (moist weight) of *Mycobacterium tuberculosis,* strain B88. The animals were housed in an air-conditioned room in groups of 5 to 8 and were maintained on a diet of Purina rabbit pellets, supplemented with fresh greens and tap water. Groups of guinea pigs were sacrificed at intervals following inoculation. Two days prior to autopsy, the animals were again skin-tested. Blood samples were obtained by cardiac puncture under ether anesthesia. Skin tests were read and extent of macroscopic tuberculosis estimated by procedures previously reported(4). Chemical and hematologic determinations were made by methods described earlier(5).

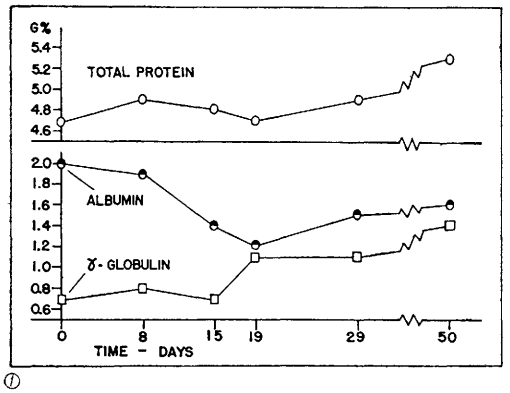
Electrophoretic analyses were carried out under conditions previously described(6) except that a current of 7.5 Ma was employed. Serum samples of 0.01 and 0.03 ml were used for protein and glycoprotein analyses, respectively. Strips were stained for protein with bromphenol blue, and for glycoprotein with the periodic acid-Schiff reagents. Absolute concentrations were determined by reference to total protein and total glycoprotein levels. Analysis of the data was made by standard statistical procedures, employing the *t* test (7).

Results. General, pathologic and hematologic data are presented in Table I. The inoculum employed caused an early conversion of dermal sensitivity and progressive disease. Significant decreases in hemoglobin concentrations were observed in the groups with the greatest amount of disease. Hematocrit levels exhibited some fluctuations. Alterations in electrophoretic patterns, however, cannot be attributed to changes in plasma volume.

Results of electrophoretic analyses are summarized in Fig. 1-4. The earliest significant change in protein patterns was the increase in α_1-globulin in the 8-day group. A further increase was noted 15 days post-inoculation, and the fraction remained significantly elevated throughout the experiment. Albumin levels were significantly decreased 15 days post-infection and remained so. Significant increases in γ-globulin concentrations were first observed in the 19-day group. Further elevation of the fraction was found in the 50-day group which resulted in a significant hyperproteinemia.

A significant increase in total glycoprotein was observed 8 days post-infection due to elevation of the protein-bound carbohydrates of the α-globulins. Maximal changes in protein-bound carbohydrates had occurred 15 days post-infection, α- and β-globulin fractions accounting for 90% of the total. γ-globulin was the only serum subfraction in which polysaccharide and protein curves paralleled each other.

Discussion. The current study has demonstrated that serum protein and glycoprotein changes previously observed in chronic



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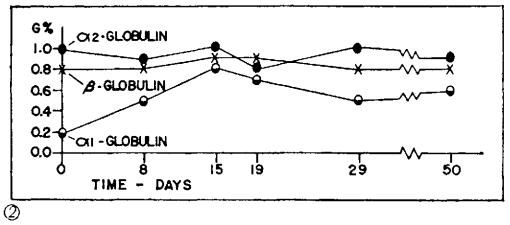

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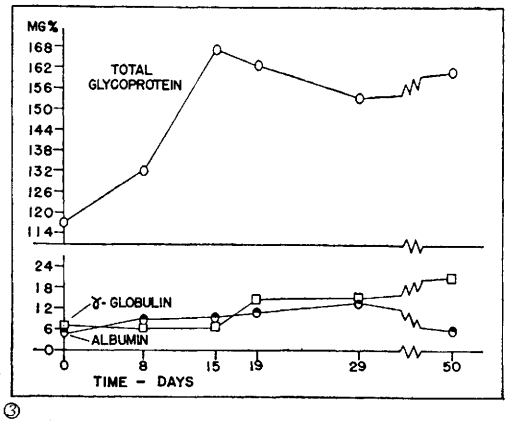

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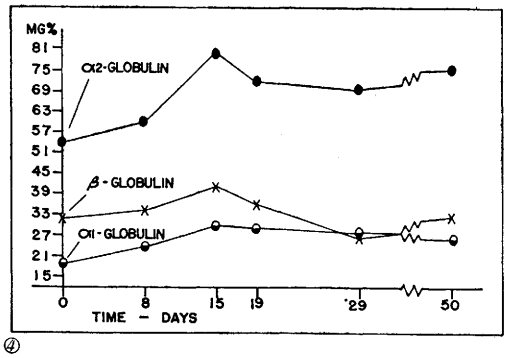

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FIG. 1 and 2. Alterations in serum protein patterns in early experimental tuberculosis.

FIG. 3 and 4. Alterations in serum protein-bound carbohydrate patterns in early experimental tuberculosis.

○, Total ●, α_2-globulin
◓, Albumin ×, β-globulin
◒, α_1-globulin □, γ-globulin

infections occur quite early in the course of the disease. With the exception of increases in the components of the γ-globulin fraction, major alterations in other subfractions of serum had occurred 15 days following infection. The lack of further changes, notwithstanding a progressive increase in disease, strongly suggests that the observations may reflect primarily systemic manifestations of the inflammatory response. The hyperproteinemia previously noted in well-established experimental disease(8) was found to occur approximately 2 months following infection and was due to increase in γ-globulin levels. In contrast to the findings in clinical tuberculosis(1,2), the protein components of the α_2-globulin fraction were little affected by the disease.

Summary. The systemic effects of acute experimental tuberculosis as reflected by alterations in concentration and distribution of the proteins and protein-bound carbohydrates of serum were investigated by filter paper electrophoresis. Electrophoretic analyses were performed on serum samples from groups of adult female guinea pigs sacrificed 8, 15, 19, 29, and 50 days following inoculation with 0.1 mg *M. tuberculosis*. Significant increases occurred in total serum glycoprotein levels and in the protein component of the α_1-globulin fraction 8 days post-infection. A marked elevation of the polysaccharide moiety of the α_2-globulin fraction and a significant decline in albumin protein was observed 15 days following infection. With the exception of significant increases in the components of the γ-globulin fraction in the latter stages of the study, major alterations in the other subfractions of serum had occurred by 15 days post-infection. It is suggested that the early alterations in electrophoretic patterns represent primarily systemic manifestations of the inflammatory response.

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Some Factors which Influence the Catalytic Activity of Pancreatic Cholesterol Esterase. (26091)

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Extracts of beef pancreas catalyze a rapid synthesis and hydrolysis of cholesteryl oleate (1,2,3). Differences in some of the requirements for optimal activity of the two reactions have been noted(3). The influence of pH, electrolyte concentration, and β-sitosterol upon catalytic activity of the enzyme is considered further in the present report.

Methods and materials. Preparation of substrates and enzyme and procedure for manometric estimation of cholesterol esterase activity have been described(1). Briefly, the composition of the standard test substrate for synthesis was 0.04 M cholesterol or other sterol, 0.12 M lithium oleate, and 0.02 M cholic acid; for hydrolysis it was 0.07 M cholesteryl oleate, 0.014 M cholic acid, and 0.035 M lithium oleate. The reaction mixture contained substrate, $NaHCO_3$, and $(NH_4)_2SO_4$ in amounts indicated with the data of each experiment. The enzyme was a 10% aqueous or 0.01 M NaCl extract of acetone-dried beef pancreas clarified by filtration. The protein content ranged from 15 to 20 mg per ml(4). Approximately 0.3 ml was used in each reaction vessel. The reaction was conducted in conventional Warburg flasks at 37°C. After appropriate periods of gassing with CO_2 and temperature equilibration, the reaction was started by pouring the enzyme solution from the side-arm into the main compartment. The first reading was made 3 minutes later, to allow for non-specific gas exchange, and thereafter at desired intervals. An increase in the pressure of CO_2 occurs during ester hydrolysis; this is indicated by a plus sign. The pressure of CO_2 decreases during ester synthesis; this is shown by a minus sign. Results are reported either as relative activities or as μl CO_2 exchanged, *i.e.* released (+) or absorbed (−), per unit time.

Results. The effect of pH on rate of synthesis and hydrolysis of cholesteryl oleate at the concentration of electrolyte which is optimal for each reaction is seen in Fig. 1. It is evident that the optima are distinct and that at the optimal pH for each reaction the opposite reaction would not occur or its rate would be negligible. At intermediate values of pH both reactions take place although at reduced rates. Nearly 50% of the maximal rate at optimal pH occurs in the vicinity of pH 6.5. This pH is taken to be the single optimum for both reactions.

The data of Fig. 2 show the effect of electrolyte concentration at optimal pH for each reaction. It is seen that the concentration of electrolyte which is optimal for synthesis is inadequate for hydrolysis. Addition of electrolyte even in amounts slightly greater than required for optimal synthetic activity causes immediate precipitation of the substrate for synthesis, resulting in diminished reaction rate. Extent of decline in rate depends upon completeness of precipitation,